Clin. exp. Immunol. (1973) **14,** 541–553.

IMMUNE RESPONSIVENESS TO *MYCOBACTERIUM LEPRAE* AND OTHER MYCOBACTERIAL ANTIGENS THROUGHOUT THE CLINICAL AND HISTOPATHOLOGICAL SPECTRUM OF LEPROSY

B. MYRVANG, T. GODAL, D. S. RIDLEY, S. S. FRÖLAND AND Y. K. SONG

Armauer Hansen Research Institute (AHRI), Addis Ababa; Hospital for Tropical Diseases, London; Institute of Immunology and Rheumatology, Rikshospitalet, Oslo, and All Africa Leprosy and Rehabilitation Training Centre (ALERT), Addis Ababa*

(*Received* 19 *December* 1972)

SUMMARY

Immunological responsiveness was studied throughout the clinical and histopathological spectrum of leprosy (Ridley–Jopling scale) by the methods of lymphocyte transformation, leucocyte migration inhibition and delayed skin hypersensitivity,

The response to *Mycobacterium leprae* showed by all methods a continuous decrease from strong responses in the polar tuberculoid (TT) group to virtually negative responses in the polar lepromatous (LL) group. There was a good agreement between the *in vitro* methods and the lepromin skin test, giving support to the latter as a useful tool in the evaluation of immune responsiveness to *M. leprae* in leprosy patients.

The immune response to BCG and PPD on the other hand, decreased only slightly towards the lepromatous pole of the spectrum, confirming the high degree of specificity of the immune defect in lepromatous leprosy. Patients grouped histologically as subpolar tuberculoid (TT/BT) reacted particularly strongly to BCG and PPD.

As it is likely that the methods used mainly measured T-lymphocyte function, the clinicopathological manifestations of leprosy appear to reflect the strength of the cellular immune response against *M. leprae*. Thus the findings give strong support to the concept of a host-determined, immunological diseases pectrum as expressed in the Ridley–Jopling classification.

INTRODUCTION

Many infectious diseases show extensive variation in clinical and histopathological findings. In recent years it has become quite clear that this variability is determined by factors related to host resistance rather than to metabolic or other variations of the causative organisms (Turk & Bryceson, 1971). This is particularly apparent in leprosy.

* Affiliated with the University of Bergen, Bergen, Norway.

Correspondence: Dr B. Myrvang, Institute for Experimental Medical Research, University of Oslo, Ullevål Hospital, Oslo 1, Norway.

Ridley & Jopling (1966) categorized leprosy patients into a spectrum according to clinical and pathological evidence of immune responsiveness to *Mycobacterium leprae*. So far, it has not been possible to obtain direct immunological support for the concept of a spectrum because no method except the lepromin test has been available to determine the immune response to *M. leprae* itself. Since the lepromin test has been found positive in a high proportion of individuals not exposed to leprosy, the immunological interpretation of the lepromin test has remained uncertain (Rees, 1964). The establishment of lymphocyte transformation (Bullock & Fasal, 1971; Godal *et al.*, 1971) and leucocyte migration inhibition (Godal *et al.*, 1972) as methods for the study of immune response to *M. leprae* has provided new opportunities to investigate the concept of an immunological spectrum in leprosy.

In the present study patients throughout the Ridley–Jopling scale have been examined by the methods of lymphocyte transformation, leucocyte migration inhibition and delayed skin hypersensitivity. The findings give strong support for the immunological basis of the Ridley–Jopling classification and the concept of a host-determined, immunological disease-spectrum in leprosy.

MATERIALS AND METHODS

Patients

One hundred and thirty-five leprosy patients at the All Africa Leprosy and Rehabilitation Training Centre (ALERT) took part in the study. The great majority were untreated. Only patients with skin lesions were accepted for the study. Clinical diagnoses were based on clinical findings and skin smear bacterial indices (Ridley, 1964). A skin biopsy from a carefully selected skin lesion was taken from most patients. Histological examination was done without information about the patient's clinical or immunological status. Patients diagnosed clinically or histologically as indeterminate leprosy (Currie, 1961) were excluded from this study.

Based on the criteria of Ridley & Jopling (1966) and Ridley & Waters (1969), the patients were clinically and histologically classified into the following seven groups: TT, polar tuberculoid; TT/BT, subpolar tuberculoid; BT, borderline tuberculoid; BB, borderline; BL, borderline lepromatous; LI, indefinite lepromatous; LL, polar lepromatous.

The TT/BT subgroup has not previously been described but patients that were histologically intermediate between TT and BT were identified separately in this study mainly because they were relatively numerous. The two principal features used in the differentiation of these groups were the degree of erosion of the epidermis by the granuloma, and the number of lymphocytes present. Merely as a consequence of this histological recognition a corresponding clinical group was introduced and provisionally defined as being composed of patients with either more than four classical tuberculoid lesions or with less than four lesions without typical tuberculoid or indeterminate characteristics.

Antigens

M. leprae was obtained from skin biopsies from heavily infected patients with lepromatous leprosy. The preparation of bacilli from the tissues has been described elsewhere (Godal *et al.*, 1971). The harvests from several biopsies were pooled and stored in suitable portions at $-70°C$ until used.

For the preparation of lepromin, bacilli were isolated as above, autoclaved and adjusted

to the WHO standard (1970) of $1{\cdot}6 \times 10^8$ acid-fast bacilli/ml saline preserved with 0·5% phenol.

Lyophilized BCG (Glaxo) was suspended in tissue culture medium, quantitated, and stored in the same way as *M. leprae*.

PPD (Evans Medical Ltd, Liverpool) was diluted in phosphate buffered saline (pH = 7·4) to give a final concentration of 50 units/ml.

Leucocyte transformation

The method of lymphocyte transformation has been described in a previous paper (Godal *et al.*, 1971).

Briefly, leucocytes were cultured in tissue culture medium containing 20% human serum from individuals without known exposure to leprosy. An antigen concentration of 4×10^6 bacilli/ml was used.

Incorporation of tritiated thymidine into DNA was measured by adding 2 μCi ^{3}H-thymidine to triplicates of *M. leprae*—stimulated and control cultures 18 hr before harvesting as described by Fröland & Natvig (1970). At the stage when the precipitates were dissolved in methanol, the cultures were sent from Addis Ababa to Oslo by air mail for counting. The ^{3}H-thymidine uptake was expressed as the ratio between the average uptake in antigen containing and control cultures (T:C ratio).

Cultures (in duplicates) for morphological quantitation of the lymphocyte blastogenosis were harvested after 7 days of cultivation. The test was in a majority of cases read blind.

Leucocyte migration inhibition

The technique originally introduced by Söborg & Bendixen (1967) was used with minor modifications as described elsewhere (Godal *et al.*, 1972).

The leucocytes were cultured in medium containing 10% horse serum. *M. leprae* was used in a concentration of $7{\cdot}5 \times 10^7$ bacilli/ml medium. The results were expressed as

$$\text{Migration index} = \frac{\text{Average area of migration with antigen}}{\text{Average area of migration without antigen}}.$$

The average area calculated was in each case based on the migration areas from at least six capillary tubes. In order to get positive values, percentage inhibition of migration = 100 − migration index × 100 was used for the calculation of correlation coefficients.

Skin tests

The skin tests were always performed after blood had been drawn for the *in vitro* tests. 0·1 ml of lepromin (i.e. $1{\cdot}6 \times 10^7$ bacilli) and PPD (i.e. 5 units) was injected intradermally into the volar surface of the forearms.

The reactions were measured as the widest transverse diameter of induration at 48 and 72 hr, and the lepromin reaction again after 3 and 4 weeks when the presence of ulceration also was noted. Both the early (Fernandez) and the late (Mitsuda) lepromin reaction were converted into five grades recommended by WHO (1953).

All injections and readings were done by the same person.

Statistical analysis

Standard deviation and correlation coefficient were used to express variance and covariance. The significance of difference in the proportion of responders in different patient

categories was measured by the χ^2 test, using graphs prepared by Miss M. V. Musset, Statistical Services Section, National Institute for Medical Research, London, and based on Mainland tables (Mainland, Herrara & Sutcliffe, 1956).

RESULTS

M. leprae-induced lymphocyte transformation was evaluated morphologically in 127 patients classified clinically and in 108 patients classified histologically. The corresponding figures were 40 and 42 for ^{3}H-thymidine incorporation, and for the leucocyte migration test 75 and 71.

The mean *in vitro* responses to *M. leprae* in relation to the clinical and histopathological diagnoses of the patients are shown in Fig. 1 (morphological lymphocyte transformation), Fig. 2 (^{3}H-thymidine uptake) and Fig. 3 (leucocyte migration inhibition). All methods gave the same result of a continuous decrease in immune response from the TT to the LL end of the spectrum. The shape of the histogram was independent of whether the grouping of the patients was based on clinical or histological examination, although there was a complete agreement on clinical and histological classification in only 51% of the patients. The decline from TT to BL was rather sharp, unlike the small difference found between the BL,

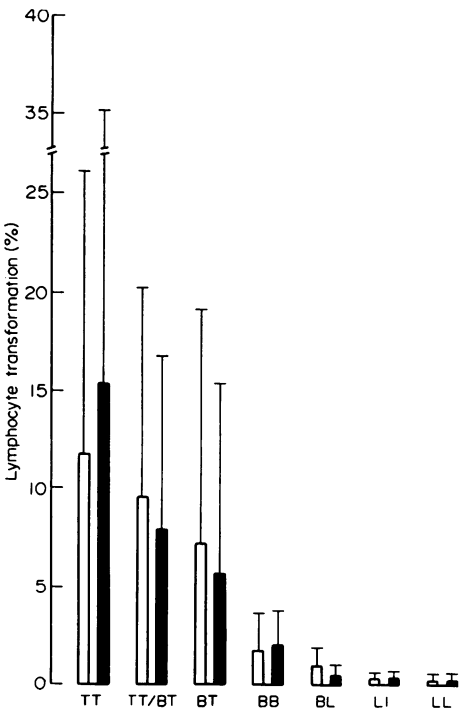

FIG. 1. Morphological lymphocyte transformation to *M. leprae* throughout the clinical and histopathological spectrum of leprosy. Bars show mean values, and lines on bars standard deviation. (□) Clinical classification. Number of observations: TT 22, TT/BT 15, BT 37, BB 8, LI 8, LL 29. (■) Histological classification. Number of observations: TT 13, TT/BT 22, BT 31, BB 4, BL 8, LI 8, LL 17.

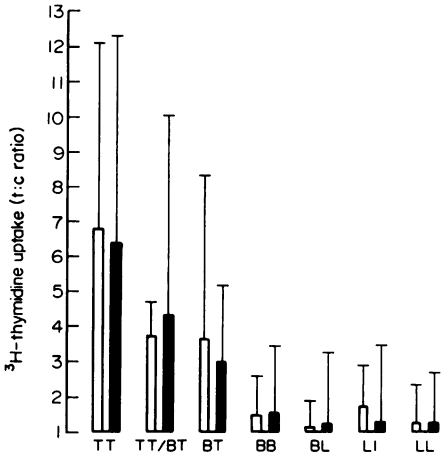

FIG. 2. ^{3}H-thymidine uptake of *M. leprae*-stimulated leucocyte cultures throughout the clinical and histopathological spectrum of leprosy. Bars show mean values, and lines on bars standard deviation. (□) Clinical classification. Number of observations: TT 6, TT/BT 4, BT 9, BB 4, LI 2, LL 11. (■) Histological classification. Number of observations: TT 4, TT/BT 6, BT 12, BB 2, BL 5, LI 6, LL 7.

LI and LL groups. Thus the decrease in mean blastogenic response within the range TT to BL was from 11·79 to 0·88% in the clinical spectrum and from 15·40 to 0·38 in the histological spectrum. The BL values differed only slightly from those of the LL group where 0·15% and 0·18% transformation was found in the clinical and histological groups respectively. This flattening of the immune response from BL to LL was even more obvious when transformation was quantitated as ^{3}H-thymidine incorporation. While the mean T:C

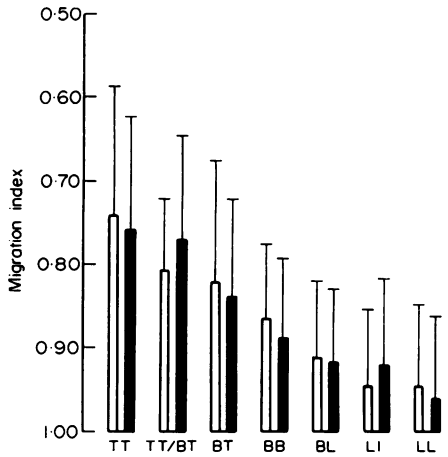

FIG. 3. *M. leprae* induced inhibition of leucocyte migration throughout the clinical and histopathological spectrum of leprosy. Bars show mean values, and lines on bars standard deviation. (□) Clinical classification. Number of observations: TT 9, TT/BT 7, BT 29, BB 9, BL 8, LI 2, LL 11. (■) Histological classification. Number of observations: TT 5, TT/BT 19, BT 23, BB 4, BL 9, LI 7, LL 4.

ratios were 6·78 and 6·39 in the clinical and histological TT group respectively, the corresponding ratios were 1·16 and 1·24 in the BB group and 1·23 and 1·25 in the LL group. The same tendency of a curved fall in responsiveness from TT to LL was also reflected in the migration indices which in the clinical spectrum were 0·74 in the TT, 0·91 in the BL and 0·96 in the LL groups and in the histological spectrum 0·76 (TT), 0·92 (BL) and 0·95 (LL).

The range of results within each patient's category (indicated as standard deviation in the histograms), was most pronounced in the TT, TT/BT and BT groups, and declined to become minimal among the lepromatous patients, specially when transformation was

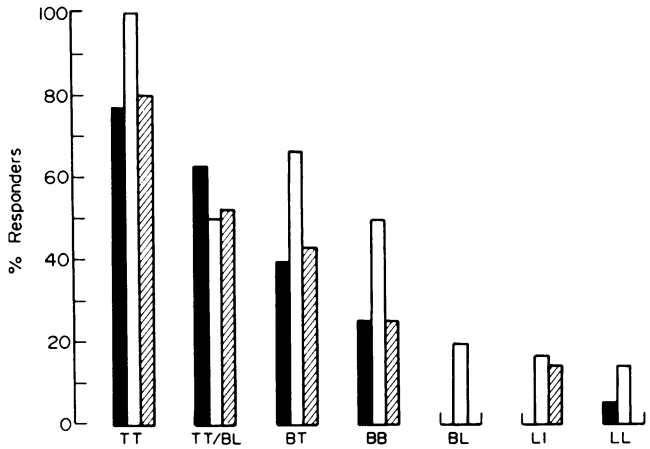

FIG. 4. Percentage of responders with the *in vitro* methods in the seven histopathological groups of leprosy patients. (■) Morphological lymphocyte transformation, (□) ^{3}H-thymidine uptake, (▨) leucocyte migration inhibition. Patients with blastogenic response above 2%, T:C ratio of >2 or migration index <0·80 were regarded as responders.

evaluated morphologically (Fig. 1). This decrease in variance of the results towards the lepromatous pole would have been more obvious also in the migration inhibition test if one had taken into account that a majority of the observed values represent various degrees of negativity.

Also when evaluating the results as percentage of responders in the seven histological categories of patients, a sharp decline was found from TT to BL, while there was roughly no difference between the BL, LI and LL groups (Fig. 4). Patients showing a blastoid transformation of above 2%, a T:C ratio of >2 or a migration index of <0·80 were regarded as responders, but the use of other thresholds did not change the basic shape of the histogram.

The coefficients of correlation between the results in the *in vitro* test varied from 0·41 to 0·65 (Table 1), being highest between morphological lymphocyte transformation and ^{3}H-thymidine incorporation. If the patients were simply grouped as responders and non-responders, the results from two tests correlated in 75–78% of the patients, while a complete correlation in all three tests was observed in 60% of those examined with all three methods.

Neither the blastogenic response nor the inhibition of leucocyte migration were statistically significantly different between adjacent groups anywhere in the spectrum. A significant difference (P <0·01) was, however, demonstrable in the responders between each tuberculoid

TABLE 1. Correlation coefficients between the response to *M. leprae* in the various tests

	Morphological lymphocyte transformation*	^{3}H-thymidine uptake†	Leucocyte migration inhibition‡	Late lepromin reaction
Early lepromin reaction	0·60	0·51	0·62	0·75
Late lepromin reaction	0·70	0·53	0·49	—
Leucocyte migration inhibition‡	0·47	0·41	—	—
^{3}H-thymidine uptake†	0·65	—	—	—

* Threshold for positivity >2% transformation.
† Threshold for positivity T:C ratio >2.
‡ Threshold for positivity < 20% inhibition.

group (TT, TT/BT, BT) and the lepromatous groups (BL, LI, LL) taken separately. The tritiated thymidine uptake displayed a similar tendency but the limited number of observations made statistical analysis inappropriate.

The early and the late lepromin reactions were recorded in seventy-nine and seventy-four patients respectively, while both reactions were read in seventy patients. The results are shown in Figs 5 and 6. The early and the late reactions had in common that they both were negative in BL, LI and LL patients and showed an increased proportion of strong responders towards the TT pole. The early lepromin reaction, like the *in vitro* tests, showed a wide range of responses within the individual groups from TT to BB in the spectrum. Thus non-responders as well as strong responders were found in all groups. In the late reaction the variation was much less pronounced, and all TT, TT/BT, BT and BB patients had positive reactions. But no specific strength of either the early or the late reaction was found to be diagnostic of any particular histological group. The results of the early and late reaction showed a considerable correlation (Fig. 7), giving a correlation coefficient of 0·75 when the results were converted into WHO grades. Comparing the mean *in vitro* response to *M. leprae* in patients grouped according to their lepromin reactivity, the *in vitro* values seemed to be better correlated with the early than with the late lepromin reaction (Fig. 8). However, calculation of correlation coefficients did not reveal any such tendency (Table 1).

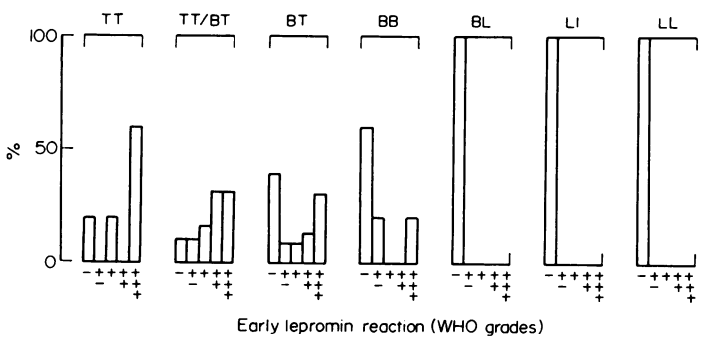

FIG. 5. Per cent responders, categorized according to strength of the early lepromin reaction, in the seven histopathological groups of leprosy patients. Number of patients in each group: TT 5, TT/BT 19, BT 23, BB 5, BL 7, LI 10, LL 10.

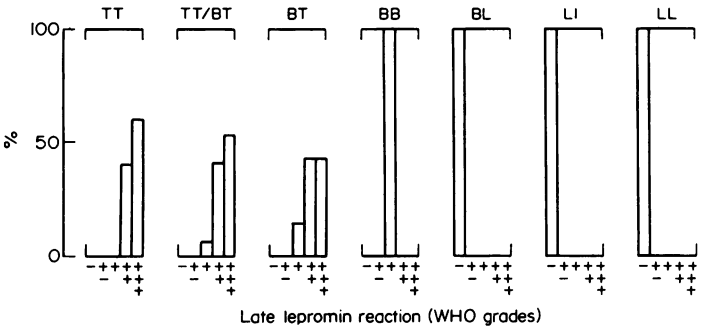

FIG. 6. Per cent responders, categorized according to strength of the late lepromin reaction, in the seven histopathological groups of leprosy patients. Number of patients in each group: TT 5, TT/BT 17, BT 21, BB 4, BL 7, LI 10, LL 10.

The composite histogram in Fig. 9 compares the mean morphological transformation to *M. leprae* and BCG (ninety-nine patients) and the mean skin reactivity to lepromin (early reaction) and PPD (sixty-nine patients). The grouping of the patients was based on histology. The response to BCG and PPD tended to decrease towards the lepromatous end of the spectrum, but not to the same extent as the response to *M. leprae* and lepromin, and there were in all groups some patients who responded quite strongly to BCG and PPD. The χ^2 test did not show any statistically significant difference in the response to BCG between any of the groups, while the remarkable strong PPD reaction in the TT/BT group was statistically significantly different from the adjacent BT groups, when a threshold of 20 mm was chosen. The coefficient of correlation between the blastogenic response to *M. leprae* and BCG was low (0·30), but somewhat higher between the early lepromin and PPD skin reactions (0·50).

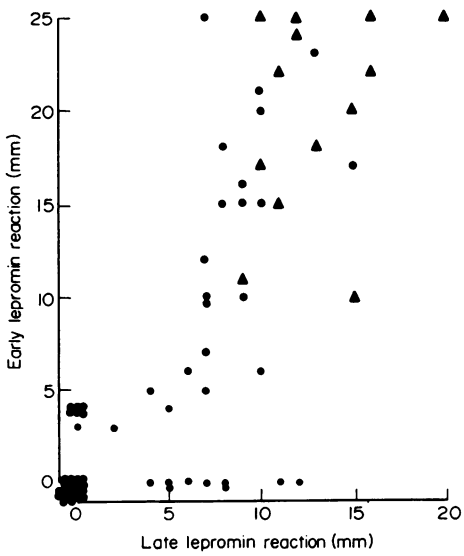

FIG. 7. Comparison between the early and late lepromin reaction in seventy leprosy patients. Triangles refer to late reactions with ulceration.

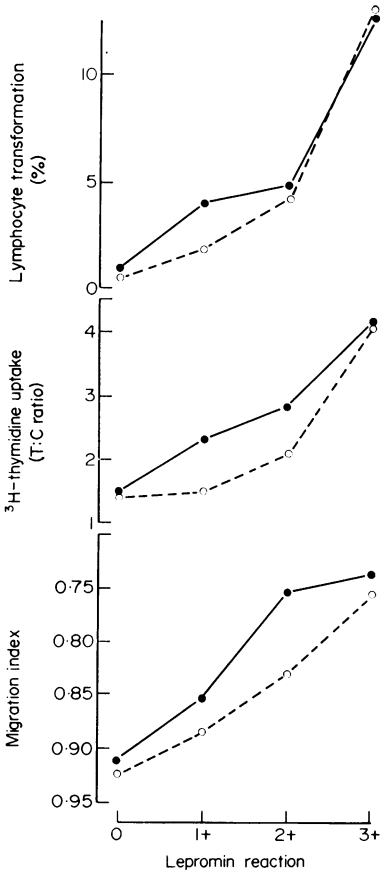

FIG. 8. Comparison between the lepromin test and the response to *M. leprae in vitro*. (●) Early lepromin reaction; (○) late lepromin reaction.

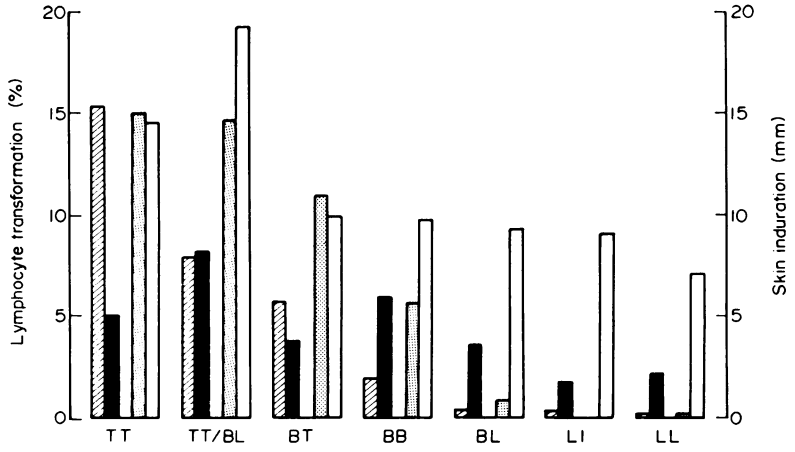

FIG. 9. Lymphocyte transformation (%) to *M. leprae* and BCG, and skin reactivity (mm) to lepromin (early reaction) and PPD throughout the histological spectrum of leprosy. (▨) Transformation to *M. leprae*; (■) transformation to BCG; (⁝⁝) early lepromin skin reaction; (□) PPD skin reaction. Number of patients tested with *M. leprae* and lepromin as in Fig. 1 and Fig. 5. Number tested with BCG and PPD: TT 11 (BCG), 6 (PPD); TT/BT 22, 17; BT 24, 18; BB 3, 5; BL 8, 9; LI 14, 7; LL 15, 7.

DISCUSSION

Immunity to intracellular parasites such as the leprosy bacillus is dependent on cell-mediated immune responsiveness (Suter & Ramseier, 1964) and expressed through T-lymphocyte-mediated activation of macrophages (Mackaness, 1969; Godal, Rees & Lamvik, 1971; Lane & Unanue, 1972). Based on clinical and pathological examination, Ridley & Jopling (1966) suggested that leprosy patients may be categorized according to immunity, i.e. cell-mediated immunity to *M. leprae*. The immunological basis for this classification has already found support in pathological studies on leprosy lymph nodes (Turk & Waters, 1971) and in experimental studies. Normal CBA mice developed after infection with *M. leprae* lesions with borderline features (Rees *et al.*, 1969), while mice immunologically suppressed by whole-body irradiation and thymectomy (Rees *et al.*, 1967) or thymectomy + antilymphocyte serum (Gaugas, 1968) developed 'lepromatous' leprosy.

Further support for the concept of an immunologically determined disease spectrum was found in the present study. A continuously decreasing response to *M. leprae* was found from the tuberculoid to the lepromatous pole of the Ridley–Jopling scale by the methods of lymphocyte transformation and leucocyte migration inhibition.

The method of leucocyte migration inhibition appears at present to be the most promising test for the evaluation of cell-mediated immunity *in vitro* as it has been found to correlate with delayed hypersensitivity in various test systems (Söborg, 1967; Rosenberg & David, 1970; Federlin *et al.*, 1971).

The finding of a similar decrease in immune response through the leprosy spectrum both by the method of leucocyte migration inhibition and that of lymphocyte transformation may seem somewhat surprising since the phenomenon of transformation is common both to T and B lymphocytes, and since lepromatous patients have been found to produce large amounts of antimycobacterial antibodies also reacting with *M. leprae* itself (Estrada-Parra, 1972). However, two factors may be considered in this connection. (1) Whole *M. leprae* organisms were used as antigen. Thus, the immune response to surface antigens may only have been measured. Since both tuberculoid and lepromatous sera have failed to block *M. leprae*-induced transformation of tuberculoid lymphocytes (Godal *et al.*, 1972) the antigen (or antigens) involved in the lymphocyte transformation test may preferentially stimulate T cells. (2) A majority of human blood lymphocytes appear to be T cells (Fröland & Natvig, 1972; Jondal, Holm & Wigzell, 1972). T cells, but not B cells are dependent on being present in peripheral blood in order to carry out their function. These considerations make it likely that the lymphocyte transformation of peripheral blood lymphocytes in the present study have mainly been a measure of T cell function.

The fall in *in vitro* responsiveness from TT to LL did not follow a straight line, but appeared to be curved when the distances between the groups were made equal. Thus, borderline patients responded less than expected with a linear correlation. This was particularly apparent in the lymphocyte transformation test. Moreover, a high proportion of borderline patients were negative in *in vitro* tests (Fig. 4). These findings do not correspond to the histological picture in which evidence of immunity (i.e. lymphocyte infiltration) and epithelioid cell formation usually may be found. Although this discrepancy may in part be due to technical variation as discussed below, one other explanation may be considered. Since borderline patients have a considerable number of bacilli in their lesions, it is possible

that *M. leprae*-reactive lymphocytes are rapidly removed from the circulation in many of these patients. Thus the situation may be analogous to some observations in humoral immunity where antibodies apparently are so rapidly absorbed by antigens in the tissues, that they are not demonstrable in serum (Milde & Tönder, 1968; Lerner, Glassock & Dixon, 1967).

Considerable variation was found within each group tested, particularly the strongest responding groups, both by the method of lymphocyte transformation and that of leucocyte migration inhibition. The reason for this variability remains uncertain. Care was taken to standardize the tests as far as possible by using pools of normal human serum and pools of antigens. However, the variation was undoubtedly in part of a technical nature as considerable variation was observed between duplicate or triplicate cultures. An additional factor related to the method of thymidine incorporation was the transportation from Addis Ababa to Oslo which made two extra transfers of culture precipitates necessary. Repeated testing of patients in reaction (Godal *et al.*, 1973) has shown that considerable changes in *in vitro* responsiveness do take place without a corresponding shift in histological or clinical picture. Thus, the number of circulating antigen-reactive lymphocytes are likely to fluctuate in leprosy patients for reasons still to be determined.

There was a fairly good agreement between all the three *in vitro* tests used in that 60% of the patients were either positive or negative by all the three methods. The method of thymidine uptake and morphological determination of lymphocyte transformation showed as expected the highest level of quantitative correlation as expressed by the coefficient of correlation. Several factors may account for the lower coefficient of correlation (Table 1) found between the methods of lymphocyte transformation and leucocyte migration inhibition. Besides being technically quite different, the methods are measuring two different events following antigenic stimulation, i.e. cellular division and liberation of molecular mediators. Although these two phenomena usually are thought to be associated, evidence has been presented that a mycobacterial fraction may induce synthesis of molecular mediators without an accompanying blastogenesis (Chaparas *et al.*, 1970) and, as mentioned above, lymphocyte transformation is not a feature of T cells only, but also a part of B cell responsiveness.

It is interesting to note how well the *in vitro* tests correlated with both the early and late lepromin reaction. These findings lend support to the lepromin test as a useful tool in the evaluation of the immune status of leprosy patients, when other methods are not available. It is also apparent from Figs 7 and 8 that the late lepromin reaction is more sensitive than the early reaction and the *in vitro* tests. This is one factor among others, that may contribute to making the late lepromin test positive in individuals who have not been exposed to the leprosy bacillus, but perhaps to cross-reacting antigens only (Turk & Bryceson, 1971).

In contrast to the non-responsiveness to *M. leprae* of the lepromatous groups, good responsiveness was found to BCG (*in vitro*) and PPD (*in vivo*) throughout the Ridley–Jopling scale (Fig. 9). The tendency to weaker responsiveness in the lepromatous as compared with the tuberculoid group may be related to the fact that a majority of the patients studied were untreated as Godal *et al.* (1972) found that treated lepromatous patients responded very well to BCG *in vitro*.

It is interesting to note that no significant difference was observed between LL and LI patients in their responsiveness to mycobacterial antigens. This finding challenges the question of whether there is a major immunological difference between LL and LI patients

(Ridley & Waters, 1969) and seems to underline the continuity of the leprosy spectrum at the lepromatous end.

The strong response to both BCG and PPD of the TT/BT group is also noteworthy, and the PPD reactions constituted a bell-shaped distribution which is characteristic of tuberculous infection (WHO, 1955). Since *M. tuberculosis* infection and BCG vaccination apparently may induce lepra reactions (Wade, 1960; Godal *et al.*, 1973), and many patients with lepra reactions reveal a histological picture of TT/BT, the strong responses to BCG and PPD in the TT/BT group may be due to tuberculous infection in BT patients which has transferred them into the TT/BT group.

ACKNOWLEDGMENT

We thank Miss Monica Löfgren for excellent technical assistance.

REFERENCES

BULLOCK, W.E. & FASAL, P. (1971) Studies of immune mechanisms in leprosy. III. The role of cellular and humoral factors in impairment of the *in vitro* immune response. *J. Immunol.* **106,** 888.

CHAPARAS, S.D., THOR, D.E., GODFREY, H.P., BAER, H. & HEDRICK, S.R. (1970) Tuberculin-active carbohydrate that induces inhibition of macrophage migration but not lymphocyte transformation. *Science,* **170,** 637.

CURRIE, G. (1961) Macular leprosy in Central Africa with special reference to the 'maculoid' (dimorphous) form. *Int. J. Leprosy,* **29,** 473.

ESTRADA-PARRA, S. (1972) Immunochemical identification of a defined antigen of *Mycobacterium leprae. Infect. Immunity,* **5,** 258.

FEDERLIN, K., MAINI, R.N., RUSSELL, A.S. & DUMONDE, D.C. (1971) A micro-method for peripheral leucocyte migration in tuberculin sensitivity. *J. clin. Path.* **24,** 533.

FRÖLAND, S.S. & NATVIG, J.B. (1970) Effect of polyspecific rabbit anti-immunoglobulin antisera on human lymphocytes *in vitro. Int. Arch. Allergy,* **39,** 121.

FRÖLAND, S.S. & NATVIG, J.B. (1972) Surface-bound immunoglobulin on lymphocytes from normal and immunodeficient humans. *Scand. J. Immunol.* **1,** 1.

GAUGAS, J.M. (1968) Enhancing effect of antilymphocytic globulin on human leprosy infection in thymectomized mice. *Nature (Lond.),* **220,** 1246.

GODAL, T., MYKLESTAD, B., SAMUEL, D.R. & MYRVANG, B. (1971) Characterization of the cellular immune defect in lepromatous leprosy: a specific lack of circulating *Mycobacterium leprae*-reactive lymphocytes. *Clin. exp. Immunol.* **9,** 821.

GODAL, T., MYRVANG, B., FRÖLAND, S.S., SHAO, J. & MELAKU, G. (1972) Evidence that the mechanism of immunological tolerance ('central failure') is operative in the lack of host resistance in lepromatous leprosy. *Scand. J. Immunol.* **1,** 311.

GODAL, T., MYRVANG, B., SAMUEL, D.R., ROSS, W.F. & LÖFGREN, M. (1973) Mechanism of 'reactions' in borderline tuberculoid (BT) leprosy. *Acta path. microbiol. scand.* Sect. A. Suppl. **236,** 45.

GODAL, T., REES, R.J.W. & LAMVIK, J.O. (1971) Lymphocyte-mediated modification of blood-derived macrophage function *in vitro*; inhibition of growth of intracellular mycobacteria with lymphokines. *Clin. exp. Immunol.* **8,** 625.

JONDAL, M., HOLM, G. & WIGZELL, H. (1972) Surface markers on human T and B lymphocytes. I. A large population of lymphocytes forming nonimmune rosettes with sheep red blood cells. *J. exp. Med.* **136,** 207.

LANE, F.C. & UNANUE, E.R. (1972) Requirement of thymus (T) lymphocytes for resistance to listeriosis. *J. exp. Med.* **135,** 1104.

LERNER, R.A., GLASSOCK, R.J. & DIXON, F.J. (1967) The role of antiglomerular basement membrane antibody in the pathogenesis of human glomerulonephritis. *J. exp. Med.* **126,** 989.

MACKANESS, G.B. (1969) The influences of immunologically committed lymphoid cells on macrophage activity *in vivo*. *J. exp. Med.* **129,** 973.

MAINLAND, D., HERRARA, L. & SUTCLIFFE, M.I. (1956) *Statistical Tables for use with Binomial Samples.* New York University College of Medicine.

MILDE, E.J. & TÖNDER, O. (1968) Demonstration of rheumatoid factor in tissue by mixed agglutination with tissue sections. *Arthr. and Rheum.* **11,** 537.

REES, R.J.W. (1964) The significance of the lepromin reaction in man. *Progr. Allergy*, **8,** 224.

REES, R.J.W., WATERS, M.F.R., WEDDELL, A.G.M. & PALMER, E. (1967) Experimental lepromatous leprosy. *Nature* (*Lond.*), **215,** 599.

REES, R.J.W., WEDDELL, A.G.M., PALMER, E. & PEARSON, J.M.H. (1969) Human leprosy in normal mice. *Brit. med. J.* **3,** 216.

RIDLEY, D.S. (1964) Appendix III. *Leprosy in Theory and Practice* (Ed. by R. G. Cochrane and T. F. Davey). John Wright & Sons Ltd, Bristol.

RIDLEY, D.S. & JOPLING, W.H. (1966) Classification of leprosy according to immunity. A five group system. *Int. J. Leprosy*, **34,** 255.

RIDLEY, D.S. & WATERS, M.F.R. (1969) Significance of variations within the lepromatous group. *Leprosy Rev.* **40,** 143.

ROSENBERG, S.A. & DAVID, J.R. (1970) Inhibition of leukocyte migration: an evaluation of this *in vitro* assay of delayed hypersensitivity in man to a soluble antigen. *J. Immunol.* **105,** 1447.

SÖBORG, M. (1967) *In vitro* detection of cellular hypersensitivity in man. Specific migration inhibition of white blood cells from brucella-positive persons. *Acta med. scand.* **182,** 167.

SÖBORG, M. & BENDIXEN, G. (1967) Human lymphocyte migration as a parameter of hypersensitivity. *Acta med. scand.* **181,** 247.

SUTER, E. & RAMSEIER, H. (1964) Cellular reactions in infection. *Advanc. Immunol.* **4,** 117.

TURK, J.L. & BRYCESON, A.D.M. (1971) Immunological phenomena in leprosy and related diseases. *Advanc. Immunol.* **13,** 209.

TURK, J.L. & WATERS, M.F.R. (1971) Immunological significance of changes in lymph nodes across the leprosy spectrum. *Clin. exp. Immunol.* **8,** 363.

WADE, H.W. (1960) BCG induced activations. *Int. J. Leprosy*, **28,** 179.

WHO, Technical Report Series No. 71. (1953) *Expert Committee on Leprosy.* First Report, Geneva.

WHO, Technical Report Series No. 459. (1970) *Expert Committee on Leprosy.* Fourth Report, Geneva.

WHO Tuberculosis Research Office (1955) Further studies of geographic variation in naturally acquired tuberculin sensitivity. *Bull. Wld Hlth Org.* **22,** 63.